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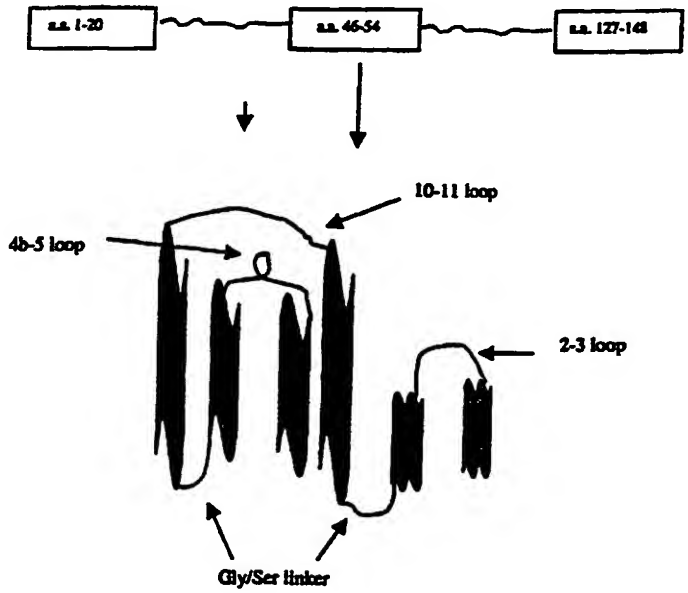
(54) Title: FimH ADHESIN-BASED VACCINES



(57) Abstract: Purified polypeptides formed from amino acid sequences corresponding to domains derived from the adhesin protein, FimH, are disclosed. Also disclosed are compositions containing these novel polypeptides for use as vaccines and for generating specific antibodies for use in treatment and/or prevention of diseases, such as urinary tract infections, caused by bacteria of the family *enterobacteriaceae*, from which FimH is derived.

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FimH ADHESIN-BASED VACCINES

This application claims the priority of U.S. Provisional Application Serial No. 60/144,016, filed 15 July 1999, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates generally to the field of novel polypeptides and polypeptide-like structures, having immunogenic activity and to vaccines comprising such structures as well as to antibodies generated in response to such structures.

BACKGROUND OF THE INVENTION

Many kinds of bacterial infections begin with attachment of bacteria to cellular surfaces present on the host. For example, in bacterial infections caused by *enterobacteria*, such as *Escherichia coli*, infection begins with colonization of mucosal surfaces by the bacteria. Such attachment is facilitated by the presence on the surfaces of the bacteria of structures referred to as pili, or fibrillae, or fimbriae. For example, in gram negative bacteria, such as *E. coli*, type 1 pili, which are adhesive fibers expressed in most bacteria of the *Enterobacteriaceae* family, facilitate the adhesive qualities of bacteria that often lead to colonization and infection of various tissues of the host animal, especially on mucosal surfaces. Such adhesion to epithelial cell surfaces is facilitated by the presence in the pilus of a protein called an "adhesin," of which FimH is an example.

Pili contain a short fibrillar tip structure composed of FimG, FimF and

FimH, attached to a rod-lik structure composed of FimA. More specifically, FimH mediates binding to mannose-oligosaccharides present on mucosal surfaces. The presence of such pili therefore plays a critical role in bacterial infection.

E. coli is the most common pathogen of the urinary tract, accounting for greater than 85% of cases of asymptomatic bacteriuria, acute cystitis and acute pyelonephritis, as well as greater than 60% of recurrent cystitis, and at least 35% of recurrent pyelonephritis infections. Because of the high incidence, continued persistence, and significant expense associated with *E. coli* urinary tract infections, there is a need for a prophylactic vaccine to reduce susceptibility to diseases such as this.

Because colonization of mucosal epithelium, such as in the urinary tract, is widely accepted as a prerequisite to infection, the disruption or prevention of pilus-mediated attachment of bacteria, such as *E. coli*, to urinary epithelia is expected to prevent or retard the development of urinary tract infections.

For example, type 1 pili are thought to be important in initiating colonization of the bladder and inducing cystitis, whereas another type of pili, called P pili, are thought to play a role in ascending infections and the ensuing pyelonephritis.

Pili are heteropolymeric structures that are composed of several different structural proteins required for pilus assembly. Type 1 pili-carrying bacteria recognize and bind to D-mannose in glycolipids and glycoproteins, for example, in bladder epithelial cells. Proteins forming the pili therefore make good candidates for vaccines. [see: Choudhury et al, X-ray Structure of the FimC-FimH Chaperone-Adhesin Complex from Uropathogenic *E. coli*, *Science* **285**, 1061 (1999); Sauer et al, Structural Basis of Chaperone Function and Pilus Biogenesis, *Science* **285**, 1058 (1999); Barnhart et al., PapD-like Chaperones Provide the Missing Information for Folding of Pilin

Proteins, *Proc. Natl. Acad. Sci. USA,* **10,** 1073/pnas.130183897 (publish d online June 20, 2000), the disclosures of all of which references are hereby incorporated by ref rence in their entirety].

A major disadvantage to pilus-based vaccines has been the fact that the major immunodominant components of pilus fibers are often antigenically highly variable and therefore afford protection against only a limited number of bacterial strains. Conversely, pilus associated adhesins, such as FimH, are highly conserved proteins among different species and strains of bacteria. FimH is also highly conserved not only among uropathogenic strains of *E. coli*, but also among a wide range of gram-negative bacteria. For example, all *Enterobacteriacea* produce FimH. Thus, vaccines incorporating antigenic portions of FimH should exhibit a broad spectrum of protection.

Attempts have been made to localize the domain of FimH capable of receptor recognition by generating translational fusion proteins of disparate (i.e., contiguous) regions of the FimH protein. Antibodies directed against the different fragments succeeded in narrowing the region of interest to about the first 25 amino acids at the N-terminal end of the FimH molecule. [See Thankavel et al., *J. Clin. Invest.* **100**:1123-1136 (1997), the disclosure of which is hereby incorporated by reference in its entirety] Such studies have therefore served to identify a need for more specific and higher affinity antigens and vaccines capable of protection against such organisms. The invention disclosed herein addresses this need.

BRIEF SUMMARY OF THE INVENTION

The present invention is directed to a novel class of polypeptides

comprising mannose binding domains derived from adhesin molecules, such as FimH.

It is an object of the present invention to provide amino acid sequences corresponding to identified domains of adhesins like FimH, such domains derived from X-ray analysis of crystals of complexes containing FimH, including the complex containing FimH and its periplasmic chaperone, FimC, referred to as the FimC-FimH complex (or FimCH).

It is also an object of the present invention to provide novel polypeptides containing amino acid sequences corresponding to highly conserved domains of FimH, and arranged so as to form a single polypeptide structure, which structure is capable of diverse biological functions, including the ability to bind ligands such as oligosaccharides and collagen, as well as eliciting an immunogenic response when injected into an animal, such as a human.

It is a further object of the present invention to provide genetically engineered or chemically synthesized polypeptides containing at least two or more of the sequences corresponding to the domains identified according to the invention and to provide methods of using such polypeptides as immunogens for eliciting antibodies.

It is also an object of the invention to provide immunogenic compositions containing purified polypeptides, other than FimH itself, or any polypeptides containing FimH, wherein such polypeptides are comprised of portions of the FimH molecules, especially where such portions are related to the binding of mannose or collagen.

It is a still further object of the present invention to provide nucleotide sequences coding for such engineered polypeptides.

It is yet a further object of the present invention to provide nov l

polypeptides useful as vaccines for prevention and/or treatment of diseases caused by bacteria of the family *enterobacteriaceae*, as well as methods for using such vaccines to treat and/or prevent such diseases, especially in humans.

It is another object of the present invention to provide for antibodies specific for the novel polypeptides disclosed herein for use in treating diseases caused by bacteria from which the adhesin structure was derived, especially *E. coli*.

It is yet a still further object of the present invention to provide for compositions containing the novel polypeptides disclosed according to the invention and to use such compositions to facilitate the disease treatment and prevention methods disclosed herein.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows a ribbon model for the lectin-binding domain of FimH. The strands are numbered from 1-10 so that the 2-3 loop, the 4b-5 loop, and the 10-11 loop are clearly illustrated. The strands appear as arrowheads pointing away from the N-terminus of the strand (and the molecule) and toward the C-terminus of the strand (and molecule).

Figure 2 shows a preferred embodiment of the invention wherein 3 domains derived from FimH are linked together by amino acid linker sequences (composed of gly/ser pairs) to form a stable and highly conserved antigenic structure. In this embodiment, the domains are mannose-binding domains MBD-1, MBD-2 and MBD-3.

Figure 3 shows an alignment of type 1 pilin sequences to the pilin domain of FimH. The end of the lectin domain and the start of the pilin

domain in the FimH are indicated by black arrowheads above the sequences. The sequenc s of FimH, FimA, FimF and FimG are all aligned to show corresponding sequences and domains. The end of the mannos -binding lectin domain and the start of the pilin domain in FimH are indicated by vertical arrowheads above the sequences. The type 1 pilin subunits (FimA, FimF and FimG) are aligned with the pilin domain of FimH using clustal W [see: Thompson et al, *Nucleic Acids Res.* **22**, 4673 (1994)] and manually adjusted to minimize gaps in secondary structure elements. Gaps in the alignment are indicated by dots. Residue 1 of FimH is residue 22 of the precursor protein. Pilus subunits (including FimH) are expressed in the cytoplasm as pre-proteins with an amino terminal signal sequence that is cleaved during transport across the inner membrane. The first residue in FimH that is visible in our maps corresponds to Phe^{22} in the gene-derived sequence, which is the expected start of the FimH chain. To distinguish residues in the adhesin protein from residues in the chaperone, FimH residues will be denoted with an "H" and FimC residues with a "C" after the residue number. Residues involved in chaperone binding are indicated by an open circle above the residue. Residues in the carbohydrate binding pocket are boxed, with a large box marking the NH_2-terminal extensions in the pilin subunits. The conserved β-zipper motif found in all pilin subunits corresponds to the F β-strand. Secondary elements are indicated below the sequence (arrows) and residues of the same character are in blue.

Figure 4 shows β-sheet topology diagrams of the mannose binding domain (Fig. 3A) and the pilin domain (Fig. 3B) of FimH. The F strand is at the C-terminal end of the pilin domain and thus would appear at the C-terminus of the FimH molecule. Each strand depicted is readily correlated with the corresponding sequences of Figure 1 wherein the strand designation appears below the arrow indicating the residues making up that strand.

Figure 5 is a block diagram showing final Mean Channel Fluoescence (MCF) versus the different sera indicated at the bottom (absorption procedure described in text) and dilutions shown in the legend on the right. The first four (left to right) sera are controls. The pre-absorbed and

absorbed sera show the dilution profiles with left to right in each profile corresponding to the dilutions in the legend (top to bottom).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel polypeptides formed from amino acid sequences corresponding to the different domains identified within the structure of the bacterial adhesin FimH based on X-ray structural analysis of FimH when complexed with its chaperone FimC. Briefly, the crystal structure was solved using MAD data to 2.7 Å collected from selenomethionyl FimC-FimH crystals. The crystals used for the structure determination belong to the symmetry group C2 with cell dimensions a= 139.08 Å, b= 139.08 Å, c=214.49 Å, and β= 89.97 Å. The crystal structure revealed the presence of a pocket in the otherwise flat surface of the lectin domain. This pocket is large enough to accommodate a single mannose unit and is located at the tip of the domain, distal to the connection with the pilin domain. The bottom of the pocket is defined by the N-terminus of the FimH and is lined with typical carbohydrate binding side chains from Asn, Gln, and Asp residues in 3 loop regions. A molecule of C-HEGA (cyclohexylbenzoyl-N-hydroxyethyl-D-glucamide) is bound in this pocket when the crystal is examined. The latter is not a known inhibitor of FimH mannose binding but was required in the crystallization to produce useful crystals. [see: Rini, *Annu. Rev. Biophys. Bioml. Struct.* 24, 551 (1995)]

As noted above (see: Thankavel et al, 1997), previous attempts have been made to produce polypeptides containing only a single mannose-binding portion of the FimH molecule. While such attempts resulted in fusion proteins that afforded some protection against urinary tract infections (UTIs) *in vivo* (as well as *in vitro* inhibitory activity), such fusion proteins have been shown by the methods disclosed herein to have included only a single domain and which failed to exhibit any binding activity. [see

Thankavel et al (1997) at page 1132]

FimH, like most proteins, is composed of various "domains," the latter made up of amino acid sequences that form secondary structures having uniquely defined biological properties. For example, based on crystal-structure analysis, residues 1-20 of FimH represent a lectin binding domain, capable of binding mannose-oligosaccharides. Such domains commonly form loop regions within the molecule and can be identified as being formed of distinct amino acid sequences. Such regions tend to be highly conserved and are therefore potential sites for immunogenic response. For example, residues 1-20 would include the 2-3 loop (meaning the loop joining strands 1 and 2 as shown in Figure 1) and part of strand 3.

In accordance with the present invention, a number of portions or domains within the FimH molecule have been identified, all of which represent highly conserved sequences of amino acids having distinct biological functions and which find use as immunogens, or vaccines, for generating antibody responses capable of protecting a host from bacterial infection and disease, wherein said diseases are caused by bacteria containing FimH as part of their pilus structures.

Generation of the amino acid sequences (plus flanking regions) to form the peptides critical to binding is therefore rendered a formality by sequence information disclosed herein, thereby providing for the formation of more specific, highly potent adhesin based vaccines. Thus, antigenic molecules with highly conserved regions are readily available for screening as vaccines.

The present invention relates to novel polypeptides comprising one or more domains as disclosed herein. Where only one domain is present, said domain will be selected from the group consisting of the sequences of SEQ ID NO: 1, 2, 3, 4, and 5. These sequences are conv niently ref rr d to as domains MBD-1, MBD-2, MBD-3, COL and MBD-C,

respectively.

Such domains are commonly involved in binding either mannose, or collagen, or both. In accordance with the methodology of the invention, mannose-binding domains within FimH have been denoted MBDs (for Mannose-Binding Domain, while the collagen binding domain has been denoted COL, and an additional domain, comprising both MBD-2 and COL has been denoted MBD-C. More specifically, MBD-1 has the sequence of SEQ ID NO: 1, MBD-2 has the sequence of SEQ ID NO: 2, MBD-3 has the sequence of SEQ ID NO: 3, COL has the sequence of SEQ ID NO: 4, and MBD-C has the sequence of SEQ ID NO: 5. As defined herein, MBD-1 is limited to including all or part of the first 20 amino acids of the FimH sequence (as defined in SEQ ID NO: 1) and does not extend any farther.

A purified polypeptide of the invention might contain only one such domain, in which case this would exclude any naturally occurring proteins from the disclosure herein since no such proteins are known to include only one of the domains disclosed herein when in a purified state. In such case, the domain could be any of the five domains just recited, with optional flanking sequences on either side of said domain so as to provide appropriate support for a more naturally occurring conformation or whatever other structural support might be need to permit the domain sequence to attain a conformation as close to natural (and functional) as possible.

Where a polypeptide of the invention comprises more than one such domain, the domains are optionally attached to each other by chemical linking structures wherein said structures are of a length that is less than the length of an oligopeptide having 25 amino acids residues (i.e., less than the length of amino acids separating any of these non-contiguous domains within a protein such as FimH). Thus, the novel polypeptides of the invention would exclude FimH itself, or any protein comprising FimH, regardless of whether or not the latter protein is

purified.

In addition, such chemical linkers may be composed of amino acids or may be composed of chemical structures, such as polymers, other than amino acids or may be composed of a mixture of amino acids and other small molecules having similar spatial dimensions to amino acids. All such types of linkers are contemplated by the invention disclosed herein. Where said chemical linkers are composed of amino acids, the preferred amino acids will be glycine and serine. The latter could include homoglycine, homoserine, or gly-ser mixtures, possibly random in sequence, or possibly alternating or some other non-random, non-alternating sequence. The nature of such sequence, as well as its length, is commonly determined by optimizing such properties with respect to the domains sought to be linked, thereby providing them with sufficient structural flexibility so as to form optimally immunogenic molecules.

When polypeptides of the present invention comprise more than one such domain, and said domains are linked by a chain of amino acids, said chain will commonly be less than 25 amino acid residues in length, preferably no more than 20 amino acid residues in length, and most preferably 5 to 10 residues in length. Of course, such linkers could be as short as 1 residue in length, or such linkers could be absent entirely and the domains linked directly to each other in a contiguous arrangement.

While the polypeptides of the present invention may contain as few as a single domain, they may also be comprised of 2, 3, or more domains, and said domains may be linked in any order.

Most preferably, the domains of the polypeptides disclosed herein will be comprised of 3 domains, most preferably MBD-1, MBD-2 and MBD-3, and the preferred embodiment will have these domains arranged in the sequence

NH_2-(MBD-1)---Linker---(MBD-2)---Linker---(MBD-3)-COOH

wherein the domains form an unbroken amino acid sequence from the N-terminal end of MBD-1 to the C-terminal end of MBD-3 and wherein the size of the linkers, or the presence of linkers at all, is optional. In the most preferred embodiment, each linker would consist of a decapeptide composed of glycine, or serine, or both, alternating or otherwise. In addition, the respective linkers within the same polypeptide may be the same or may be different; if the latter, they may differ in either length or chemical identity or both. Thus, one linker might be composed of only amino acids and the other might be some other type of chemical structure, such as an organic polymer. In the latter case, the term "polypeptide" might be more loosely defined to include a structure composed of polypeptide sequences that may or may not be continuous. Where the linkers are composed of amino acids, these may be the same or different in sequence and may be the same or different in length.

Of course, it is also contemplated by the disclosure herein that not just the order of domains within a sequence might be different but also the orientation of the amino acid sequences within a given domain might be reversed (i.e., inverted). For example, screening might show that reversing the orientation of MBD-2 within the above sequence provides for a more antigenic structure. This could be accomplished simply by reversing the sequence of the amino acids in one or more of the domains or sequences disclosed according to the invention (by direct synthesis of the polypeptides, or oligonucleotides encoding the sequences of MBD-1, MBD-2, MBD-3, MBD-C or COL) or by synthesizing a gene to be expressed inside of a suitably engineered cell).

It should be reiterated that the polypeptides of the invention comprise 1, 2, 3, or more such domains strung together like beads on a

chain. These domains may be present in any order and may include sequences in which one type of domain, for example, MBD-1, is present in multiple copies. Thus, by way of non-limiting example, the possibl 2 domain (all mannose binding) combinations might be:

NH_2-(MBD-1)---Linker---(MBD-1)-COOH

NH_2-(MBD-2)---Linker---(MBD-2)-COOH

NH_2-(MBD-3)---Linker---(MBD-3)-COOH

NH_2-(MBD-3)---Linker---(MBD-1)-COOH

NH_2-(MBD-1)---Linker---(MBD-3)-COOH

NH_2-(MBD-3)---Linker---(MBD-2)-COOH

NH_2-(MBD-2)---Linker---(MBD-3)-COOH

NH_2-(MBD-1)---Linker---(MBD-2)-COOH

NH_2-(MBD-2)---Linker---(MBD-1)-COOH

Similar combinations and permutations can readily be devised by those skilled in the art when 3, 4 or more such domains are linked to form a polypeptide of the present invention and all such combinations and permutations are expressly contemplated by the present invention.

In addition, as stated, the polypeptides of the invention may have the domains with sequences oriented in an opposite direction and still be within the invention. Of cours , such reversals of orientation in th sequences of the amino acids might require some degree of chemical

modification but all such modifications are deemed within the ordinary capabilities of those skilled in the art.

The most preferred embodiments of the present invention are polypeptides in which the arrangement of domains is:

NH_2-(MBD-1)---Linker---(MBD-2)---Linker---(MBD-3)-COOH

and wherein MBD-1 will have the amino acid sequence of SEQ ID NO:1, MBD-2 will have the amino acid sequence of SEQ ID NO: 2, and MBD-3 will have the amino acid sequence of SEQ ID NO: 3 and wherein each linker is about 10 amino acids in length, including linkers with exactly 10 amino acids in length, said amino acids being glycine, serine or a mixture of both, wherein said mixture may be alternating glycine and serine residues or may be a random mixture.

In addition, it should be kept in mind that while the domains disclosed herein are all derived from highly conserved domains identified in FimH, it is deemed well within the ordinary skill in the biochemical art to find amino acid replacements for one or more of the amino acids disclosed in the domain sequences of the invention. Consequently, such amino acid replacements are deemed within the present invention if said replacements number no more than at most 20% of the amino acids in said domain sequence. More particularly, this would mean the replacement of no more than 4 amino acids in MBD-1, nor more than 2 amino acids in MBD-2, no more than 4 amino acids in MBD-3, and no more than 6 amino acids in COL.

In addition, the present invention would also encompass any polypeptides having the mannos -binding domains, or oth r domains, of the invention with an arrangement, as limited by the disclosure herein, of

the domains of the invention and wherein said domains have amino acid sequences, individually or in combination, that are at least 80% homologous (i.e., have at least 80% sequence id ntity) with the domain sequences disclosed herein.

In accordance with the present invention, the term "percent identity" or "percent identical," when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The Percent Identity is then determined according to the following formula:

$$\text{Percent Identity} = 100\,[1-(C/R)]$$

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence wherein (i) each base or amino acid in the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, constitutes a difference; and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

If an alignment exists between the Compared Sequence and the Reference Sequence for which the percent identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum percent identity to the Reference Sequence even though alignments may exist in which the hereinabove calculated Percent Identity is less than the specified Percent

Identity.

In addition, the sequences of the domains disclosed herein, in order to be contemplated by the present invention, need not necessarily have the same lengths as the corresponding domain sequences found in native FimH or as disclosed in the sequence listing but may be either shorter or longer than such sequences. Of course, longer sequences will normally comprise the domains disclosed herein and therefore are expressly contemplated by the present invention. Conversely, shorter sequences may also be contemplated by the present invention if they contain sufficiently large and functional segments or fragments of the domain sequences disclosed herein. Thus, such shorter segments may comprise fragments of the domain sequences disclosed herein that contain at least 80% of the amino acids of said sequences.

For MBD-1, containing the 20 amino acids of SEQ ID NO: 1, a sequence comprising any 16-mer (i.e., 80%) sequence within SEQ ID NO: 1 is deemed within the present invention as well as any smaller segments adding up to such a 16-mer. For example, residues 1-8, 2-17, 3-18, 4-19, and 5-20 would all represent segments or fragments within the MBD-1 sequence disclosed herein. The same would apply to the sequences of the other domains disclosed herein. Thus, any 7-mer within the sequence of MBD-2 would be expressly contemplated by the present invention.

As used herein, the terms "portion," "segment," and "fragment," refer to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin or chymotrypsin, the oligopeptides resulting from such treatment would represent portions, segments or fragments of the starting polypeptide.

Also as used herein, the term "FimH" r fers to the FimH protein as

found in type 1 pili of cells of the family *enterobacteriaceae*, and including the protein with the sequence of SEQ ID NO: 9 as well as proteins having sequence similarity thereto and performing the same function in bacterial cells, regardless of the bacterial cell source.

In addition, such substitutions need not be limited to replacement with other L-amino acids. Bacterial cell wall substances are known to possess some D-amino acids and, where appropriate, the sequences disclosed herein may contain one or more such D-amino acids, or even chemically modified amino acids, if this is found to result in greater immunogenicity of the polypeptides of the invention, provided that such substitutions follow the guidelines stated above for sequence homology and size.

It is also contemplated that the polypeptides of the present invention may be in isolated or purified form.

"Isolated" in the context of the present invention with respect to polypeptides (or polynucleotides) means that the material is removed from its original environment (*e.g.*, the cells used to recombinantly produce the polypeptides disclosed herein). Such peptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The recombinant and/or synthetic immunogenic polypeptides, disclosed in accordance with the present invention, will commonly be used in a "purified" form. The term "purified" does not require absolute purity; rather, it is intended as a relative definition, and can include preparations that are highly purified or preparations that are only partially purified, as those terms are understood by those of skill in the relevant art. For example, polypeptides from individual clones isolated from a cDNA library have been conventionally purified to electrophoretic

homogeneity.

For purposes of recombinantly producing the polypeptides of the invention, the term "expression product" means that polypeptide or protein that is the natural translation product of the gene and any nucleic acid sequence coding equivalents resulting from genetic code degeneracy and thus coding for the same amino acid(s).

Thus, the polypeptides of the present invention may also be present in the form of a composition. Such composition, where used for pharmaceutical purposes, will commonly have the polypeptide of the present invention suspended in a pharmacologically acceptable diluent or excipient.

The novel peptides of the invention are in no way limited to the mannose-binding domains but may also include other domains identified within the FimH structure. Thus, while amino acids 1-20 of FimH represent a natural mannose-binding domain (SEQ ID NO: 1), residues 1-15 would not be expected to bind mannose. However, the latter would represent a highly conserved region of the polypeptide easily accessible to antibodies.

In addition, other stretches of amino acids, such as residues 50-80 (SEQ ID NO: 4) of FimH, form the β-sheet-α-1-β-sheet structure of strands 5, α-1, and 6. While not expected to have mannose binding activity, it does contain a critical region involved in collagen binding activity for some uropathogenic *E. coli* strains (especially residue 62) and forms a highly conserved domain on FimH accessible to antibodies.

Such domains may be linked together in single or multiple copies just as already described for the mannose-binding domains, with optional linkers and other accessory structures necessary to maintain proper conformation.

In addition, regardless of the types of domains being linked, such

linkers may also be selected for providing properties other than proper conformation or orientation. For example, such linkers may be chosen for their ability to confer increased solubility properties on the polypeptide as a whole.

In another embodiment, the polypeptides of the invention may comprise all of the different domains identified according to the invention. In this case, it should be noted that domains MBD-2 and COL have overlapping sequences (the C-terminal 5 residues of MBD-2 are the same as the N-terminal 5 residues of COL) and thus a new arbitrary domain (MBD-C) has been identified for purposes of the present invention. This domain has the sequence of SEQ ID NO: 5.

In its native state, FimH is often associated with its characteristic chaperone, FimC, to which it binds via hydrophobic residues. Because the novel polypeptides of the invention will necessarily be devoid of these FimC-binding sequences, they are therefore expected to be much more soluble than adhesins generally and also to be more compatible with MF-59 (as well as other adjuvants) for long-term storage than is FimCH.

The present invention is also directed to polynucleotides capable of coding for the polypeptides of the invention, especially polynucleotides encoding the amino acid sequence of a preferred embodiment of the present invention as shown in SEQ ID NO: 6. Such polynucleotides therefore contain at least one coding region for the polypeptides of the present invention, which is therefore an expression product of such polynucleotides.

As used herein, the term "coding region" refers to that portion of a gene which either naturally or normally codes for the expression product of that gene in its natural genomic environment, i.e., the region coding *in vivo* for the native expression product of the gene. The coding region can be from a normal, mutated or altered gene, or can even be from a DNA sequence, or gene, wholly synthesized in the laboratory using methods

well known to those of skill in th art of DNA synthesis.

In accordance with th present invention, the term "nucl otide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the proteins provided by this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

The term "expression product" means that polypeptide or protein that is the natural translation product of the gene and any nucleic acid sequence coding equivalents resulting from genetic code degeneracy and thus coding for the same amino acid(s).

As used herein, reference to a DNA sequence includes both single stranded and double stranded DNA. Thus, the specific sequence, unless the context indicates otherwise, refers to the single strand DNA of such sequence, the duplex of such sequence with its complement (double stranded DNA) and the complement of such sequence.

Thus, in accordance with the present invention, the different domain sequences can be cloned into a phage display system, subjected to random mutagenesis, as well as site-directed mutagenesis, and panned for high affinity binders to mannose and/or collagen in a solid phase assay. Such higher affinity molecules can then be used to generate antibodies against the novel polypeptides disclosed herein.

The present invention is also directed to antibodies specific for, and antisera generated in response to, polypeptides of the invention. Such antibodies may be either polyclonal or monoclonal and may be generated, where monoclonal, from a cell, especially a hybridoma cell, by standard methods in the art. In addition, the present invention also relates to cells,

and cell lines, genetically engineered to produce such antibodies after being transfected, or otherwise transformed, so that their genomes contain, within the main chromosome or as part of a plasmid or other vector, a polynucleotide encoding the genes for an antibody specific for a polypeptide of the invention, especially where said engineered cell is a cell capable of forming and secreting a fully formed antibody, such technology being known in the art.

The present invention also relates to vectors, such as plasmids, comprising the polynucleotides of the invention, said polynucleotides encoding polypeptides disclosed herein, and wherein such vectors are useful for transforming cells and permitting said transformed cells to express the polypeptides of the invention.

The present invention also relates to cells transformed by such vectors and thereby expressing, with or without subsequent secretion thereof, the polypeptides of the invention.

The present invention is also directed to vaccines containing the polypeptides disclosed herein. Such a vaccine would comprise an immunogenically effective amount of a polypeptide of the invention. A preferred embodiment of the invention is a vaccine comprising the polypeptide having the arrangement

NH_2-(MBD-1)---Linker---(MBD-2)---Linker---(MBD-3)-COOH

wherein each of the linkers is composed of 5 to 10 amino acid residues, and most especially where the sequence of the entire structure is the sequence of SEQ ID NO: 6. The latter shows the amino acid sequence of a preferred embodiment comprising mannose-binding domains of FimH linked by glycine/serine linkers of 10 residues each between the three mannose-binding domains.

Other preferred embodiments are shown in SEQ ID NO: 7 wherein the polypeptide chain comprises MBD-1, MBD-C and MBD-3 separated by glycin /serine decapeptide linkers and in SEQ ID NO: 8 wherein collagen and mannose-binding domains of FimH are separated by decapeptide linkers composed of glycine/serine pairs.

It is an object of the present invention to utilize an immunogenic composition for a vaccine (or to produce antibodies for use as a diagnostic or as a passive vaccine) comprising a novel fusion polypeptide of the invention. In one embodiment, proteins and fragments (naturally or recombinantly produced, as well as functional analogs) of these polypeptides are contemplated.

In one embodiment, the invention disclosed herein relates to an immunogenic composition comprising a purified polypeptide, said polypeptide comprising a portion of FimH, said portion selected from the group consisting of MBD-1, MBD-2, MBD-3, COL, and MBD-C, wherein said polypeptide is other than FimH or a polypeptide comprising FimH. Thus, the purified polypeptides comprising the immunogenic compositions of the present invention do not include FimH itself, or pre-FimH, or any other polypeptide or protein comprising FimH or its preprotein. For the polypeptides that form the immunogenic compositions of the invention, the portion of FimH contained in said polypeptides may be a mannose-binding portion of FimH or a collagen-binding portion of FimH or some other well defined and highly conserved portion of the FimH molecule, wherein said FimH is the FimH found in any of the bacteria of the *enterobacteriaceae* family, such as *E. coli*.

In another aspect of the invention, an immunogenic composition according to the invention may be utilized to produce antibodies to diagnose urinary tract infections, or to produce vaccines for prophylaxis and/or treatment of such infections as well as booster vaccines to maintain a high titer of antibodies against the immunogen(s) of the immunogenic

composition.

In addition, such antibodies may be gen rated using the polypeptides of the present invention for research purposes, as a means of studying protein-lectin or collagen binding and interactions.

While other antigens have been utilized to produce antibodies for diagnosis and for the prophylaxis and/or treatment of bacterial urinary tract infections, there is a need for improved or more efficient vaccines. Such vaccines should have an improved or enhanced effect in preventing bacterial infections mediated by adhesins and pili.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers are generally added that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used.

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as adjuvants, wetting agents. or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

Vaccines are generally formulated for parenteral administration and are injected either subcutaneously or intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art. Available adjuvants for human administration include aluminum hydroxide gels.

The amount of vaccine sufficient to confer immunity to pathogenic bacteria is determined by methods well known to those skilled in the art.

This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically, the amount of vaccine to be administered will be determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or intramuscular injection, a range of 50 to 500 μg purified protein may be given.

In addition to use as vaccines, the polypeptides of the present invention, and immunogenic fragments thereof, can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

Recombinant polypeptides of the invention will commonly result from the engineering of the amino acid sequence of the domains disclosed herein with appropriate linker structures to provide for conformational flexibility and to meet stereospecific needs in generating appropriate structures for use as immunogens. This is readily accomplished by engineering the appropriate DNA sequence, inserting this sequence into a vector and then transforming the appropriate cells to express the desired polypeptides. Such an approach may then be used to produce a cell line that stably expresses the genetically engineered polypeptide.

Of course, such recombinant expression cannot be used when the chemical linkers are of a chemical nature other than that of simple chains of amino acids.

In addition, because the amino acid sequences disclosed herein are not very long, even when as many as 3 domains are used and linkers are as much as 20 amino acids in length, the polypeptides of the present invention can be readily synthesized by chemical means, especially automated means, well known in the biochemical art. Further, oligonucleotides coding for th polypeptides disclosed herein can likewise be synthesized and used to

prepar th polypeptides of the invention.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a non-human. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention. In addition, cells can be transformed with gene sequences corresponding to antibody chains containing variable

regions complementary to the polypeptides of the invention and thereby generate engineered antibodies to the polypeptides disclosed her in.

The present invention is also directed to the uses of the disclosed polypeptides as vaccines to treat diseases caused by bacterial species and to the uses of antibodies specific for the polypeptides of the invention in treating such diseases.

Thus, the present invention is directed to a method of preventing a disease in an animal at risk thereof comprising administering to said animal the vaccines according to the invention, especially where said disease is a disease caused by a bacterium of the family *enterobacteriaceae,* most especially when the bacterium is *E. coli* and most preferably where the animal is a human.

The present invention is also directed to a method of treating a disease in an animal afflicted therewith comprising administering to said animal a pharmacologically effective amount of the composition of the antibodies specific for the polypeptides disclosed herein, wherein said antibodies are suspended in a pharmacologically acceptable carrier, diluent or excipient and are present in a sufficient amount to result in amelioration of the disease condition, especially where the bacterium is of the family *enterobacteriaceae* and most especially where the bacterium is *E. coli*, and most preferably where the animal to be treated is a human.

In carrying out the procedures of the present invention it is of course to be understood that reference to particular buffers, media, reagents, cells, culture conditions and the like are not intended to be limiting, but are to be read so as to include all related materials that one of ordinary skill in the art would recognize as being of interest or value in the particular context in which that discussion is presented. For example, it is often possible to substitute one buff r system or culture medium for another and still achieve similar, if not identical, results. Those of skill in

the art will have sufficient knowledg of such systems and methodologies so as to be able, without undue experimentation, to make such substitutions as will optimally serve their purposes in using the methods and procedures disclosed herein.

The present invention will now be further described by way of the following non-limiting examples. In applying the disclosure of these examples, it should be kept clearly in mind that other and different embodiments of the present invention will no doubt suggest themselves to those of skill in the relevant art.

Example

Peptide immunogenicity

The reactivity of different sera (mouse, rabbit, and primate) was evaluated for differential reactivity with each of the different peptides, MBD-1, MBD-2, MBD-3 etc., with results in Table 1 and the serum denoted at the top of the columns (polyclonal antibodies).

The peptides tested were MBD1, MBD2, MBD3, CBD, and CHAP (MBD = mannose binding domain, CBD = chaperone binding domain, and CHAP = chaperone-pilin complex (a complex of FimC and FimH).

The peptide sequences were as follows:

MBD1-FACK

20-mer: FACKTANGTAIPIGGGSANV (SEQ ID NO: 10)

MBD2-TQIF

20-m r: TQIFCHNDYPETIDYVTLQR (SEQ ID NO: 11)

MBD3-ETIT

21-mer: ETITDYVTLQRGSAYGGVLSN (SEQ ID NO: 12)

CBD-AVLI

19-mer: AVLILRQTNNYNSDDFQFV (SEQ ID NO: 13)

CHAP-VVVP

21-mer: VVVPTGGCDVSARDVTVTLPD (SEQ ID NO: 14)

The results using sera from primate, rabbit and mouse are summarized in table 1 with experiments performed as follows: the immunogen indicated in column 1 was administered to the animal for the indicated period and dose and then a sample of the peptide placed in the test well and the serum from the corresponding animal tested for reactivity (as measured by the indicated titer). For example, under column 2, MBD-1 was the peptide tested with each of the sera from column 1 and the appropriate titers are listed under anti-MBD-1 in column 2. Thus, BL02 in column 1 indicates the first primate group and serum from this primate was tested with MBD-1 to give an anti-MBD-1 of <100.

The results showed that the most potent antisera (anti-FimH T3, rabbit ME91, immunized against the truncate FimH T3) were reactive with all 3 MBD peptides with greater reactivity than with CHAP peptide. FimH T3 is a truncate comprising a large portion of the N-terminal part of FimH. Other antisera preparations showed some reactivity with MBD peptides, in particular MBD-2. In general, however, high titer antisera are needed to detect this reactivity. Of note is that the ME91 antisera did *not* react

strongly with the CBD peptide, which is outside the mannose binding domain.

In addition, ME91 antisera (as described for Figure 5) was absorbed with each of the peptides and was tested *in vitro* for ability to inhibit binding of type 1-piliated E. coli (strain NU14) to J-82 bladder cells (human). Here, J82 represents the cells alone while the normal human rabbit and human sera acted as controls. The control sera had no antibodies to FimH and thus the ability of the bacteria to bind to bladder cells was typically high (shown by the MCF (mean channel fluorescence) in the 500 to 700 range. (for the protocol, see Langermann et al, *Science* **276**, 607-611 (1997), the disclosure of which is hereby incorporated by reference in its entirety). Here, the anti-FimH T3 serum (from rabbit ME91 of table 1 – the truncate is further described in Langermann et al (1997)) was a potent inhibitor of binding of the bacteria to bladder cells, hence the low MCF for aT3. However, when this serum was absorbed (i.e., treated with one of the peptides as disclosed herein to remove that particular specificity from the serum (which serum was, of course, polyclonal) there appeared to be no change in the dilution profile over the pre-absorbed (Figure 5). For example, the serum was absorbed with MBD-1 and the dilution profile shown above the MBD-1 absorbed entry at the bottom. The ability of each dilution to inhibit binding of bacteria to bladder cells at each dilution for the given absorbed antiserum is then shown by the indicated MCF. In general, regardless of whether serum were absorbed with MBD-1, MBD-2 or MBD-3, there was no effect (i.e. diminution) of the inhibitory titers as shown in Figure 5. Inability to remove inhibitory activity with peptide absorption suggests that polyclonal sera targets multiple domains on FimH (resulting in agglutination) and/or a need to generate conformational specific antibody responses (to the binding cleft) for functionality. Thus, the presence of any one MBD domain alone appears to be insufficient unless the appropriate conformation is achieved or that multiple pitopes ar being recognized simultaneously by the polyclonal antibodies.

The results of these absorption experiments are summarized in Table 1, in which primate, rabbit and mouse sera were used. The sera, as indicated in column 1 of the table, are as follows:

Primate sera: BL02, BL11, BL13, BLL15, BL18, BL19

Here, monkeys received FimCH vaccine + MF59 adjuvant (Chiron Corp.) (20 mcg or 100 mcg as indicated). Sera from each of these monkeys was tested for reactivity with the different FimH MBD peptides + the FimH CBD (collagen binding domain) and FimH CHAP (chaperone domain) peptides as shown. Endpoint titers to the FimH T3 molecule are shown as well in the last column. The sera tested were obtained from either 12 or 33-week bleeds, following immunization regimens as indicated in the Table.

There was no significant reactivity seen with any of the sera against any of the peptides (with the exception of BL19 sera tested against MBD-1).

Rabbit sera: 86-20, 129-12

These rabbits received FimCH + CFA/IFA at the indicated doses and time points. Sera from rabbit 86-20 showed reactivity with all three MBD peptides (MBD-2 > MBD-1 > MBD-3), but not to the control peptides. Sera from rabbit 129-12also showed reactivity to all three MBD peptides but MBD > MBD-2 > > MBD-3. This sera also reacted with the CHAP peptide (the CHAP peptide has a sequence of 3 contiguous amino acids NDY which are shared with the MBD-2 peptide, and in the MBD-2 region of FimH these 3 amino acids form contacts with mannose (see: Choudhury *et al*, above). Whether this is the reason for the cross reactivity is not known at the present time.

Rabbit serum 3063MF59 tox lot: This is a control rabbit immunized with MF59 adjuvant only (used in a toxicity study in support of the IND submission for the MEDI-516 FimCH vaccine). No reactivity was found with any of the peptides (as expected).

Rabbit serum 3090 FimCH tox lot: Rabbit used in toxicity study cited above. Received multiple, closely spaced injections of FimCH + MF59 (~ 200 µg/injection). The only significant reactivity seen was with CHAP peptide. Endpoint titers to FimH T3 however have not been evaluated so far, therefore the relevance of the MBD reactivity profile is unclear.

Rabbit 50-16wk, 200 µg FimC: Rabbit injected with FimC alone. Sera tested as negative control. No significant reactivity was observed with any of the peptides (slight reactivity seen with CBD).

Rabbit ME91: Rabbit was injected with FimHT3 truncate protein (Regimen and dose as indicated). Strong reactivity with MBD-1, MBD-2 and MBD-3 peptides and some (at least 10-fold lower) reactivity with CHAP was

observed. Slight reactivity was observed with CBD (100 fold lower than with MBD peptides).

Mouse sera: SP126-1, SP126-4, SL70-1 These mouse sera samples represent high-titer (FimHT3-specific but lower than the rabbit FimH T3 specific antiserum), from mice receiving either FimHdsc (donor strand complemented FimH – see Barhart et al (2000), above), FimHT2 (His-tag truncate of FimH) or FimHT3 (His tag truncate of FimH) immunogens (see Langermann et al (1997). No reactivity was observed with any of the peptides.

Table 1

Serum from primates, rabbits, and mice immunized with varying doses and regimens of Fim CH or Fim C Pilus proteins to test for antibody responses to peptides corresponding to the 3 mannose-binding domains and collagen-binding domain of Fim H and a negative control buried domain of Fim H with chaperone pilin molecule (CHAP)

IMMUNOGEN	Anti MBD-1	Anti MBD-2	Anti MBD-3	Anti CBD	Anti CHAP	AntiFim H T3
Primate Serum						
BL02- 12 wk, 20 ug Fim CH + 4 wk boost	<100	<100	<100	<100	<100	>12800
BL02- 33 wk, 20 ug Fim CH + 4 & 24 wk boost	<100	<100	<100	<100	<100	>409600
BL11- 12 wk, 20 ug Fim CH	<200	<100	<100	<100	<100	>51200
BL13- 33 wk, 100 ug Fim CH + 4 & 24 wk boost	<100	<100	<100	<100	<100	>25600
BL15- 33 wk, 100 ug Fim CH + 4 & 24 wk boost	<100	<100	<100	<100	<100	>51200
BL18- 33 wk, 100 ug Fim CH + 4 & 24 wk boost	<100	<100	<100	<100	<100	>102400
BL19- 33 wk, 100 ug Fim CH + 4 & 24 wk boost	<800	<100	<100	<100	<100	>102400
Rabbit Serum						
86- 20 wk, 200 ug Fim CH + 4 wk 50 ug boost	>800	>3200	>400	<100	<100	>26214400
129- 12 wk, 200 ug Fim CH + 4 & 8 wk 50 ug boost	>6400	>3200	>200	<100	>6400	>3276800
3063 MF59 tox lot	<100	<100	<100	<100	<100	ND
3090 Fim CH tox lot	<100	<100	<100	<100	>3200	ND
50- 16 wk, 200 ug Fim C + 4 wk 50 ug boost	<10	<10	<10	>320	<10	ND
ME91 - 14 wk, 200 ug Fim H T3 + 3 (50ug) boost	>20480	>20480	>20480	>320	>2560	>52428800
Mouse Serum						
SP126 -1 - 8 wk, 2 ug Fim Hdsc + 4 wk boost	<10	<10	<10	<10	<10	>102400
SP126 - 4- 8 wk, 8 ug Fim H T2 + 4 wk boost	<10	<10	<10	<10	<10	>204800
SL70-1 15 wk, 20 ug Fim H T3 + 4 & 13 wk boost	<10	<10	<10	<10	<10	>25600

ND = not done

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising a purified polypeptide, said polypeptide comprising a portion of FimH, said portion selected from the group consisting of MBD-1, MBD-2, MBD-3, COL, and MBD-C, wherein said polypeptide is other than FimH or a polypeptide comprising FimH.

2. The immunogenic composition of claim 1 wherein said portion of FimH is a mannose-binding portion of FimH.

3. The immunogenic composition of claim 1 wherein said portion of FimH is a collagen-binding portion of FimH.

4. A purified polypeptide comprising one or more domains, such that where more than one domain is present said domains are attached to each other by chemical linking structures of a length less than that of 25 amino acids.

5. The polypeptide of claim 4 wherein the chemical linkers are composed of amino acids.

6. The polypeptide of claim 4 wherein the linker is comprised of a chain of from 1 to 20 amino acids.

7. The polypeptide of claim 6 wherein the amino acids are selected from the group consisting of glycine and serine.

8. The polypeptide of claim 6 wherein said domains are selected from the group consisting or MBD-1, MBD-2, MBD-3, COL and MBD-C.

9. The polypeptide of claim 8 wherein the polypeptide contains only one domain.

10. The polypeptide of claim 8 wherein th polypeptid contains 2 domains.

11. The polypeptide of claim 8 wherein the polypeptide contains 3 domains.

12. The polypeptide of claim 8 wherein the domains are arranged in the order selected from the group consisting of NH_2-MBD-1-MBD-2—MBD-3-COOH and NH_2-MBD-1-MBD-C—MBD-3-COOH..

13. The polypeptide of claim 4 wherein the polypeptide contains only 1 domain selected from the group consisting of MBD-1, MBD-2, MBD-3, COL, and MBD-C.

14. The polypeptide of claim 11 wherein the polypeptide contains 4 domains.

15. The polypeptide of claim 14 wherein the domains are arranged in the order NH_2-MBD-1-MBD-2-COL-MBD-3-COOH.

16. The polypeptide of claim 8 wherein MBD-1 has the sequence of SEQ ID NO. 1, MBD-2 has the sequence of SEQ ID NO: 2 and MBD-3 has the sequence of SEQ ID NO: 3.

17. A polynucleotide comprising a coding region for the polypeptide of claim 13.

18. An antibody specific for a polypeptide selected from the group consisting of the polypeptides of claims 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16.

19. The antibody of claim 18 wherein said antibody is a

monoclonal antibody.

20. A composition comprising the antibody of claim 19 suspended in a pharmaceutically acceptable diluent or excipient.

21. A vaccine comprising an immunogenically effective amount of a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16.

22. A composition comprising an immunogenically active amount of a polypeptide selected from the group consisting of the polypeptides of claims 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16, wherein said polypeptide is suspended in a pharmaceutically acceptable diluent or excipient.

23. A method of preventing a disease in an animal at risk thereof comprising administering to said animal the vaccine of claim 21.

24. The method of claim 21 wherein the disease is caused by a bacterium of the family *enterobacteriaceae.*

25. The method of claim 24 wherein the bacterium is *E. coli.*

26. The method of claim 25 wherein the animal is a human.

27. A method of treating a disease in an animal afflicted therewith comprising administering to said animal a pharmacologically effective amount of the composition of claim 21.

28. The method of claim 27 wherein the disease is caused by a bacterium of the family *enterobacteriaceae.*

29. The method of claim 28 wherein the bacterium is *E. coli.*

30. The method of claim 29 wherein the animal is a human.

31. The method of claim 30 wherein the disease is a urinary tract infection.

32. The method according to claim 31 wherein the urinary tract infection is caused by *E. coli.*

Fig. 1

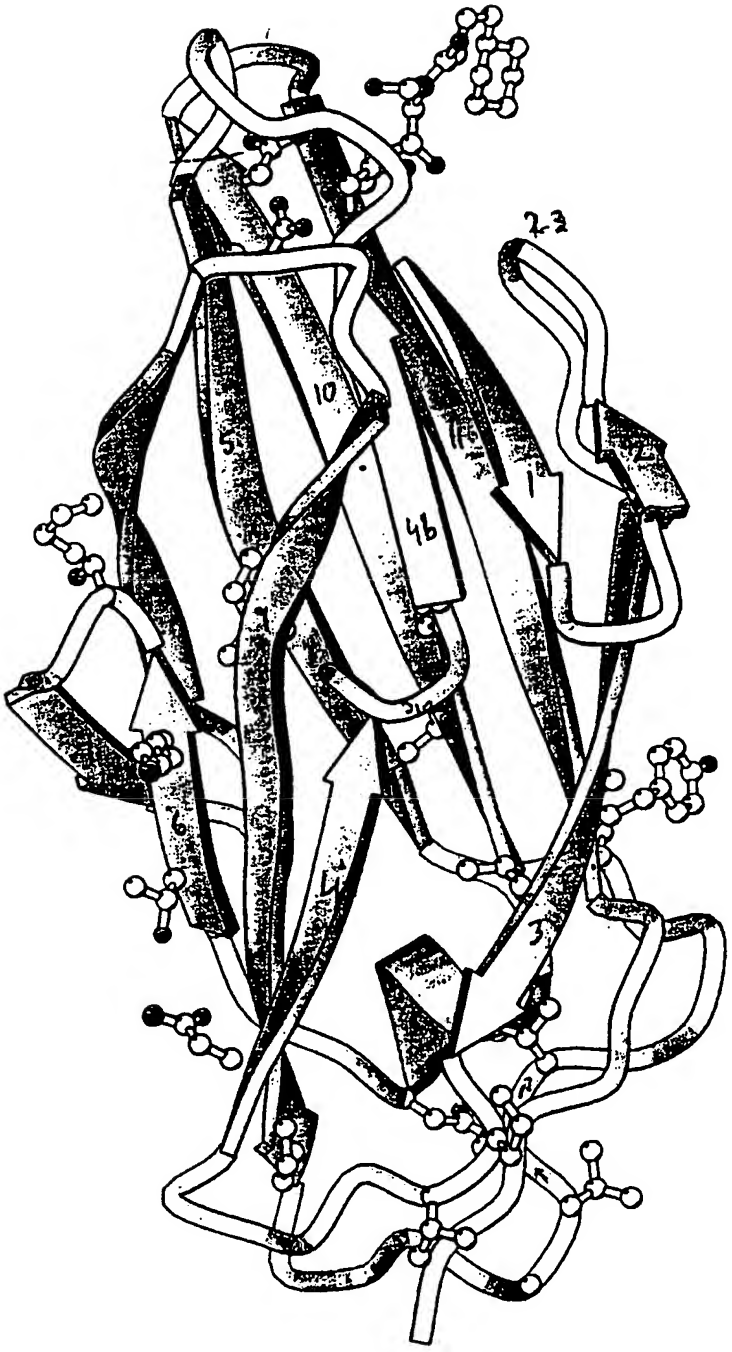
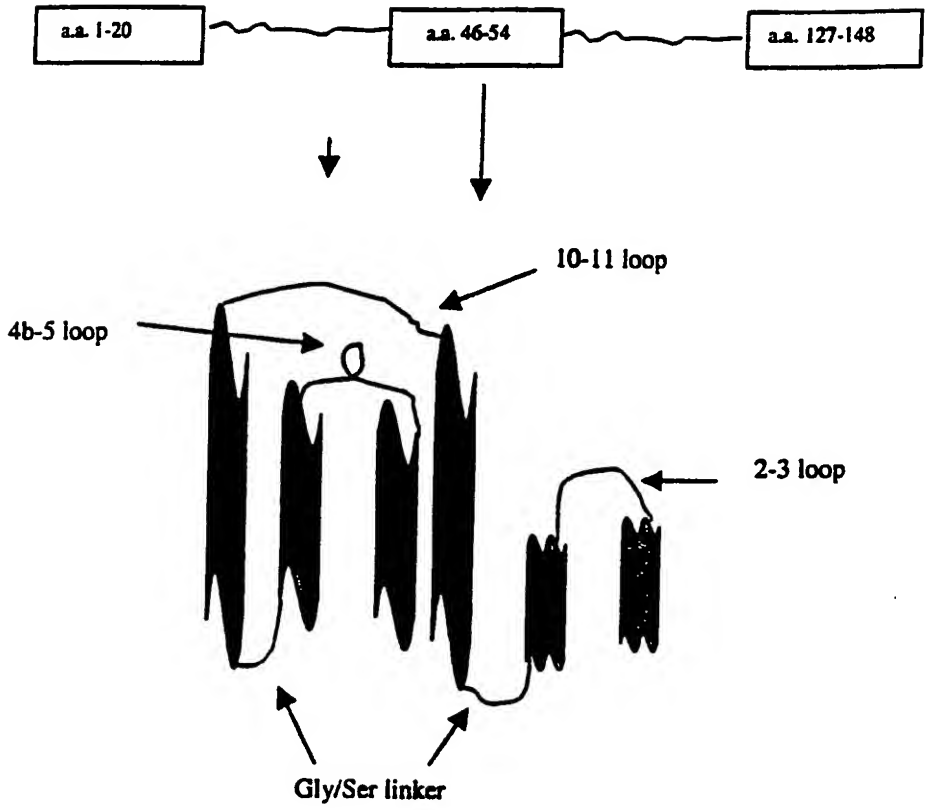


Fig. 2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Gly/Ser linker

Fig. 3

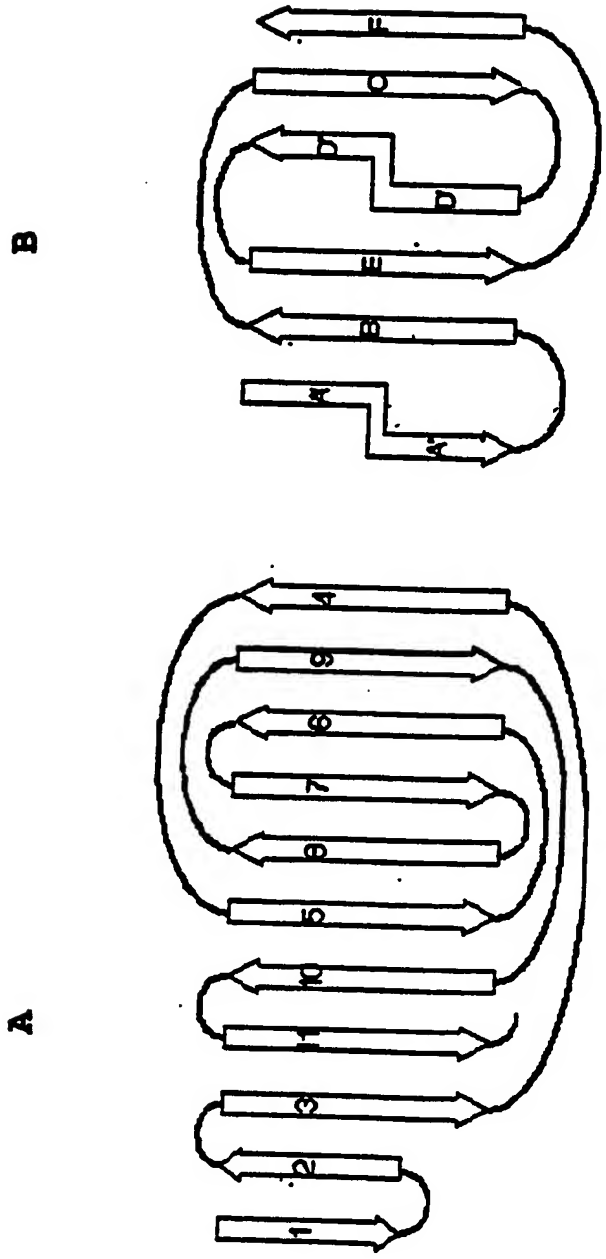
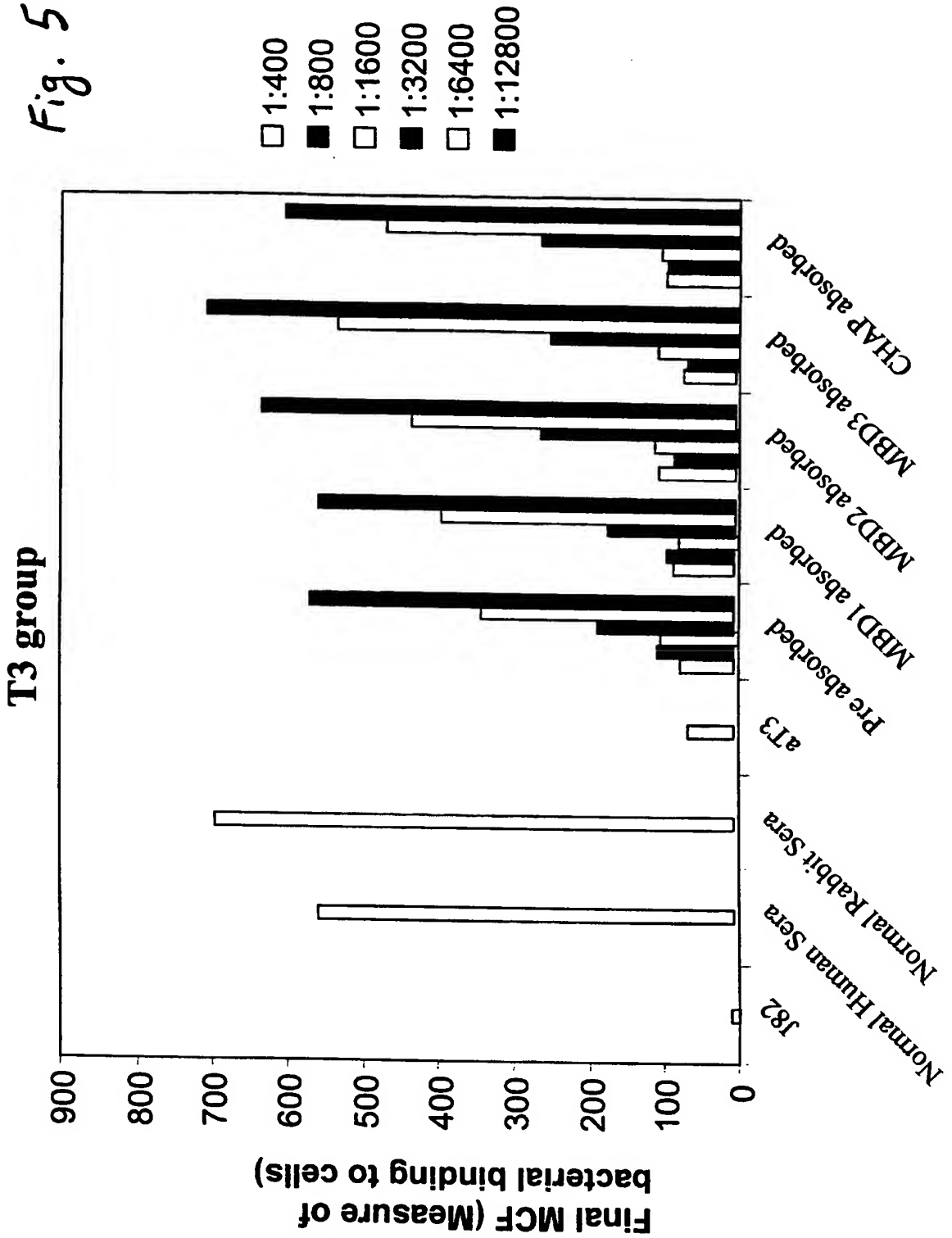


Fig. 4

Fig. 5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Normal Rabbit Sera
aT3
Pre absorbed
MBD1 absorbed
MBD2 absorbed
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CHAP absorbed

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Tyr Gly Gly Val Leu Ser Asn Phe Ser Gly Thr Val Lys Tyr Gly Ser
    50                  55                  60

Gly Ser Gly Ser Gly Ser Gly Ser Ala Val Leu Ile Leu Arg Gln Thr
65                  70                  75                  80

Asn Asn Tyr Asn Ser Asp Asp Phe Gln Phe Val Trp Asn Ile
                85                  90
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<210> 8

<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Novel polypeptide containing mannose- and collagen-binding domains of E. coli FimH separated by glycine-serine linkers (residues 21-30, 40-49, and 79-88)

<400> 8

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Phe Ala Cys Lys Thr Ala Asn Gly Thr Ala Ile Pro Ile Gly Gly Gly
 1               5                  10                  15

Ser Ala Asn Val Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Asn Asp
            20                  25                  30

Tyr Pro Glu Thr Ile Thr Asp Gly Ser Gly Ser Gly Ser Gly Ser Gly
        35                  40                  45

Ser Glu Thr Ile Thr Asp Tyr Val Thr Leu Gln Arg Gly Ser Ala Tyr
    50                  55                  60

Gly Gly Val Leu Ser Asn Phe Ser Gly Thr Val Lys Tyr Ser Gly Ser
65                  70                  75                  80

Gly Ser Gly Ser Gly Ser Gly Ser Ala Val Leu Ile Leu Arg Gln Thr
                85                  90                  95

Asn Asn Tyr Asn Ser Asp Asp Phe Gln Phe Val Trp Asn Ile
            100                 105                 110
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<210> 9
<211> 279
<212> PRT
<213> Escherichia coli

<400> 9

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Phe Ala Cys Lys Thr Ala Asn Gly Thr Ala Ile Pro Ile Gly Gly Gly
 1               5                  10                  15

Ser Ala Asn Val Tyr Val Asn Leu Ala Pro Val Val Asn Val Gly Gln
            20                  25                  30

Asn Leu Val Val Asp Leu Ser Thr Gln Ile Phe Cys His Asn Asp Tyr
        35                  40                  45
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Pro Glu Thr Ile Thr Asp Tyr Val Thr Leu Gln Arg Gly Ser Ala Tyr
50 55 60

Gly Gly Val Leu Ser Asn Phe Ser Gly Thr Val Lys Tyr Ser Gly Ser
65 70 75 80

Ser Tyr Pro Phe Pro Thr Thr Ser Glu Thr Pro Arg Val Val Tyr Asn
85 90 95

Ser Arg Thr Asp Lys Pro Trp Pro Val Ala Leu Tyr Leu Thr Pro Val
100 105 110

Ser Ser Ala Gly Gly Val Ala Ile Lys Ala Gly Ser Leu Ile Ala Val
115 120 125

Leu Ile Leu Arg Gln Thr Asn Asn Tyr Asn Ser Asp Asp Phe Gln Phe
130 135 140

Val Trp Asn Ile Tyr Ala Asn Asn Asp Val Val Val Pro Thr Gly Gly
145 150 155 160

Cys Asp Val Ser Ala Arg Asn Val Thr Val Thr Leu Pro Asp Tyr Pro
165 170 175

Gly Ser Val Pro Ile Pro Leu Thr Val Tyr Cys Ala Lys Ser Gln Asn
180 185 190

Leu Gly Tyr Tyr Leu Ser Gly Thr Thr Ala Asp Ala Gly Asn Ser Ile
195 200 205

Phe Thr Asn Thr Ala Ser Phe Ser Pro Ala Gln Gly Val Gly Val Gln
210 215 220

Leu Thr Arg Asn Gly Thr Ile Ile Pro Ala Asn Asn Thr Val Ser Leu
225 230 235 240

Gly Ala Val Gly Thr Ser Ala Val Ser Leu Gly Leu Thr Ala Asn Tyr
245 250 255

Ala Arg Thr Gly Gly Gln Val Thr Ala Gly Asn Val Gln Ser Ile Ile
260 265 270

Gly Val Thr Phe Val Tyr Gln
275

<210> 10

<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Peptide segment derived from FimH and used in immunization studies

<400> 10
Phe Ala Cys Lys Thr Ala Asn Gly Thr Ala Ile Pro Ile Gly Gly Gly
1 5 10 15

Ser Ala Asn Val
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<210> 11
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Peptide segment derived from FimH and used in immunization studies

<400> 11
Thr Gln Ile Phe Cys His Asn Asp Tyr Pro Glu Thr Ile Asp Tyr Val
1 5 10 15

Thr Leu Gln Arg
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<210> 12
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Peptide segment derived from FimH and used in immunization studies

<400> 12
Glu Thr Ile Thr Asp Tyr Val Thr Leu Gln Arg Gly Ser Ala Tyr Gly
1 5 10 15

Gly Val Leu Ser Asn
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<210> 13
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Peptide segment derived from FimH and used in immunization studies

<400> 13
Ala Val Leu Ile Leu Arg Gln Thr Asn Asn Tyr Asn Ser Asp Asp Phe
1 5 10 15

Gln Phe Val

<210> 14
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Peptide segment derived from FimH and used in immunization studies

<400> 14
Val Val Val Pro Thr Gly Gly Cys Asp Val Ser Ala Arg Asp Val Thr
1 5 10 15

Val Thr Leu Pro Asp
20

INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/US 00/19402

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/245 C07K16/12 A61K39/108 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12R A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 20657 A (GX BIOSYSTEMS AS ;SOKURENKO EVGENI VENIAMINOVIC (US); HASTY DAVID) 3 August 1995 (1995-08-03) page 2, line 17 - line 26 page 4, line 9 - line 16 page 8, line 1 - line 2 page 9, line 7 -page 15, line 22 claims 1-83; figures 4,5; example 1; table 1 -/--	1-32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2000	27/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

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INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/US 00/19402

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THANKAVEL ET AL: "Localization of a domain in the FimH Adhesin of Escherichia coli Type 1 fimbriae capable of receptor recognition and use of a domain-specific antibody to confer protection against experimental urinary tract infection" JOURNAL OF CLINICAL INVESTIGATION,US,NEW YORK, NY, vol. 100, no. 5, September 1997 (1997-09), pages 1123-1136, XP002109165 ISSN: 0021-9738 cited in the application figures 1,4; tables 2-4 ---	1,2,4-32
X	POUTTU ET AL.: "Amino acid residue Ala-62 in the FimH fimbrial adhesin is critical for the adhesiveness of meningitis-associated Escherichia coli to collagens" MOLECULAR MICROBIOLOGY, vol. 31, no. 6, March 1999 (1999-03), pages 1747-1757, XP000971304 page 1750, column 1, last paragraph -page 1751, column 2 ---	3
A	SCHEMBRI ET AL.: "Linker insertion analysis of the FimH adhesin of type 1 fimbriae in an Escherichia coli fimH-null background" FEMS MICROBIOLOGY LETTERS, vol. 137, 1996, pages 257-263, XP000971155 abstract page 257, column 1 -page 258, column 1 table 1 ---	1-32
P,X	CHOUDHURY D ET AL: "X-ray structure of the FimC-FimH chaperone-adhesin complex from uropathogenic Escherichia coli" SCIENCE, 13 AUG. 1999, AMERICAN ASSOC. ADV. SCI, USA, vol. 285, no. 5430, pages 1061-1066, XP002154985 ISSN: 0036-8075 cited in the application figures 2,3 -----	1-32

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. ial Application No
PCT/US 00/19402

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9520657 A	03-08-1995	AU 1532795 A	15-08-1995
		CA 2180726 A	03-08-1995
		EP 0738325 A	23-10-1996